Undersea Biomedical Research, Vol. 2, No. 3, September 1975

See 1473

Supportive evidence for altered platelet function in the dived rat

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Frattali, V., M. Quesada, and R. Robertson. 1975. Supportive evidence for altered platelet function in the dived rat. Undersea Biomed. Res. 2(3):167-172.–A study was conducted on the changes in platelet function and platelet count in the Sprague-Dawley rat induced by a bends-producing N_2-O_2 compression-decompression cycle. In those instances where mild to moderate cases of decompression sickness were produced, a decrease in platelet reactivity to ADP-induced aggregation occurred immediately postdive along with an increase in inhibition of aggregation by prostaglandin E_1. Both effects returned to control levels 24 hours postdive. In moderately affected animals, platelet counts were lower than normal 24 hours postdive but were similar to control values 72 hours postdive. These results tend to support current hypotheses regarding the etiological relationship between disseminated intravascular coagulation and decompression sickness as a function of bubble nucleation.

decompression collapse syndrome	platelet aggregation
decompression sickness	platelet count
platelets	rat

A better understanding of the sequela of decompression collapse syndrome (DCS), also known as decompression sickness, has emerged from a number of investigations conducted within the last few years. Upon reviewing the available literature up to the late 1960's, Holland (1969) suggested that the pathogenesis of DCS is explicable in large measure if disseminated intravascular coagulation (DIC) is considered as a contributing factor. More recently, a review by Philp (1974) summarized the experimental evidence for a positive relationship between DIC and DCS following compression-decompression in animals, as well as in man. Among the effects of diving on various cellular and molecular blood components either in the presence or absence of overt DCS, the evidence for an interaction between platelets and bubbles appears firmly established. Geller (1941) first suggested the possibility of an interaction between bubbles and platelets, but offered no supportive evidence. Jacobs and Stewart (1942) reported on the envelopment of bubbles by platelet aggregates in blood flowing from the severed tails of rats subjected to explosive decompression, and Clay (1963) observed the formation of thrombi and platelet aggregates in histological sections of lung tissue from severely decompressed dogs.

In a series of articles, Philp and associates reported that the peripheral platelet count in rats was decreased following decompression (Philp 1964; Philp, Gowdey, and Prasad 1967), and the central role of platelets in the formation of microthrombi in rats and other animals was demonstrated (Philp, Schacham, and Gowdey 1971). A number of investigators have noted a loss of platelets in humans following compression-decompression (Sicardi 1970; Bennett and Gray 1971; Philp, Ackles, Inwood, Livingstone, Achimastos, Binns-Smith, and

Radomski 1972). Further, Martin and Nichols (1972), Philp, Freeman, Francey, and Ackles (1974) and Philp, Inwood, Ackles, and Radomski (1974) observed that peripheral platelet count continued to fall as late as 72 hours postdive when losses averaged about 30% of predive values. Interestingly, prophylactic use of antiplatelet drugs was found to diminish the thrombocytopenic response, platelet adhesiveness, and sensitivity to ADP-induced aggregation (Philp, Inwood, et al. 1974).

Based on some of the earlier animal experiments mentioned above of Philp and associates linking DIC with DCS, we launched on a study to assess the prophylactic and therapeutic value of several drugs on DCS using the albino rat as a model. Such counter-hemostatic drugs as Persantine (Boehringer Ingelheim, Ltd., a brand of dipyridamole), prostaglandin E_1 (PGE_1), phenylbutazone, and acetylsalicylic acid (ASA) were selected because of their known ability to inhibit either platelet aggregation or the platelet release reaction elicited by plasmin with animal as well as human platelets (Niewiarowski, Senyi, and Gillies 1973). Recently, however, the results of a thorough study by Inwood (1973) have become available wherein a number of questions that we posed at the outset of our study have been satisfactorily answered. Hence, the purpose of this communication is to corroborate some of Inwood's findings and to report other information which supplements his work and supports his conclusions.

EXPERIMENTAL METHODS

All rats used in this study were of the NMRI:0(SD) variety, a Sprague-Dawley strain bred by the Veterinary Medical Sciences Department of the Naval Medical Research Institute. Male animals weighing approximately 200 g were selected because the characteristic reaction to DCS by members of this population has been observed frequently in this laboratory. A sample of PGE_1 was obtained as a gift from The Upjohn Company; likewise, Persantine (registered tradename), a brand of dipyridamole, was received as an aqueous solution prepared for intravenous use by Boehringer Ingelheim, Ltd.

Dives were conducted in a Bethlehem Corporation Chamber, Model 18365HP. The animal retainer used to contain rats was a duplicate of that described elsewhere (Sallee and Adams 1970). Dive schedules I and III given by Sallee and Adams (1970) were followed without change. Both schedules, which require about 70 min from start of compression to initiation of decompression, utilize N_2-O_2 gas mixtures and are bends-producing dives for the type of rodent used in this study.

Body weights were obtained within 1 to 2 hours prior to a dive. In experiments involving drug tests, animals weighing approximately 200 g were randomly selected and administered the drug or a saline solution within 30 min prior to a dive. Usually, a group of 16 animals, equally divided between treatment and control, was dived at the same time. Upon removal from the chamber after a dive, surviving rats were observed for 45 min for symptoms of DCS. Each animal was scored for severity of symptoms according to the following criteria: 0, no symptoms; 1, rapid ventilation; 2, paresis and chokes; 3, dead. For comparative purposes with other classification schemes, a "score 1" animal is considered to possess mild symptoms, and a "score 2" animal would possess moderate to severe symptoms.

In experiments involving the effects of diving on platelet aggregation, dived animals and nondived controls were anesthetized with diethyl ether and blood was withdrawn from the descending aorta into a syringe containing a known volume of 3.8% sodium citrate. The final volume ratio of blood to citrate solution was 9 : 1. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained by differential centrifugation of citrated blood according

to established techniques (Born and Cross 1963). A Chrono-Log aggregometer attached to a strip chart recorder was used to measure aggregation. The upper and lower limits for full scale deflection of 100 units on the recorder were established with a water blank and a standard sulfosalicylate-albumin suspension. Each PRP suspension was adjusted to a baseline turbidity of 45 units by addition of an appropriate volume of PPP, thus producing a suspension of the order of 2.9×10^5 platelets/mm^3. A constant temperature of 37° was maintained in the cuvette chamber of the aggregometer, and all suspensions were stirred at 1100 rpm. The extent of ADP-induced aggregation was determined from the pen displacement of the recorder following addition of 20 μl of an ADP solution, 25 μg/ml, and 10 μl of saline to 0.5 ml of a PRP suspension. To measure percent inhibition of ADP-induced aggregation, a 10 μl aliquot of a 100 ng/ml solution of PGE_1 replaced the saline solution and was mixed with PRP 30 sec prior to addition of ADP.

In the experiments involving repeat platelet counts on the same animal, 20 μl of blood was taken up from a fresh incision in an animal's tail and processed for counting according to instructions for the Unopette (Becton-Dickson) pipetting system.

RESULTS AND DISCUSSION

Table 1 gives the results of ADP-induced aggregation on platelets obtained from animals exposed to our most rapid decompression schedule. In comparison to the scoring system for DCS as given by Inwood (1973), the two sets of dived animals in Table 1 would be classified in the "mild" or "moderate" DCS categories. We did not obtain any information on dead animals or those with severe DCS because of the difficulties encountered in drawing a sufficient volume of hypercoagulable blood from the relatively small, young rats in these population groups. Nevertheless, where Inwood (1973) showed a statistically significant difference in platelet aggregation between control rats and test rats with moderate to severe

TABLE 1

Postdive platelet aggregation in rats

	Dived animals	Controls	*P*
ADP-induced aggregation, immediate postdive	19.6 ± 1.3 (40)	24.6 ± 0.8 (41)	$<.005$
ADP-induced aggregation, 24 hours postdive	22.6 ± 1.1 (20)	23.3 ± 1.1 (24)	NS
Percent inhibition of ADP-induced aggregation by PGE_1, immediate postdive	67.0 ± 3.6 (40)	42.1 ± 3.0 (41)	$<.001$
Percent inhibition of ADP-induced aggregation by PGE_1, 24 hours postdive	40.9 ± 4.5 (20)	46.0 ± 3.0 (24)	NS

All data are presented as Mean ± SEM (*n*). A unit of measure of aggregation corresponds to a deflection of 0.1 inch by the pen of a strip chart recorder connected to the output leads of a photometric aggregometer. Mean body weights for all four groups of animals were between 177 and 182 g.

DCS, the data in Table 1 extend the same conclusion to indicate that the circumstances are similar in less debilitating cases of DCS. This effect on platelet aggregation is a transient phenomenon since there is no apparent difference between control and dived animals 24 hours postdive. To further analyze the drop in platelet reactivity, the degree of inhibition elicited by PGE_1 was assessed in parallel with the aggregation experiments. As seen in Table 1, PGE_1 is a more effective inhibitor of ADP-induced aggregation with platelets drawn immediately postdive than it is with samples obtained 24 hours later. These observations tend to lend support to the conclusion that intact platelets from symptomatic animals are somewhat refractory to aggregating agents after a provocative dive and that this refractoriness is lost after a matter-of hours.

Figure 1 is a composite of data collected on the time course for changes in platelet count with three populations of rats. The results showing no change in platelet counts immediately postdive are comparable to information provided by Inwood (1973) wherein counts for unaffected, and mildly or moderately affected rats are similar to control values. However, Figure 1 also shows that the peripheral platelet levels for the two categories of dived rats are depressed 24 hours later and gradually return to near-normal levels. These results are similar to observations made in man by Martin and Nichols (1972) and Philp, Inwood et al. (1974).

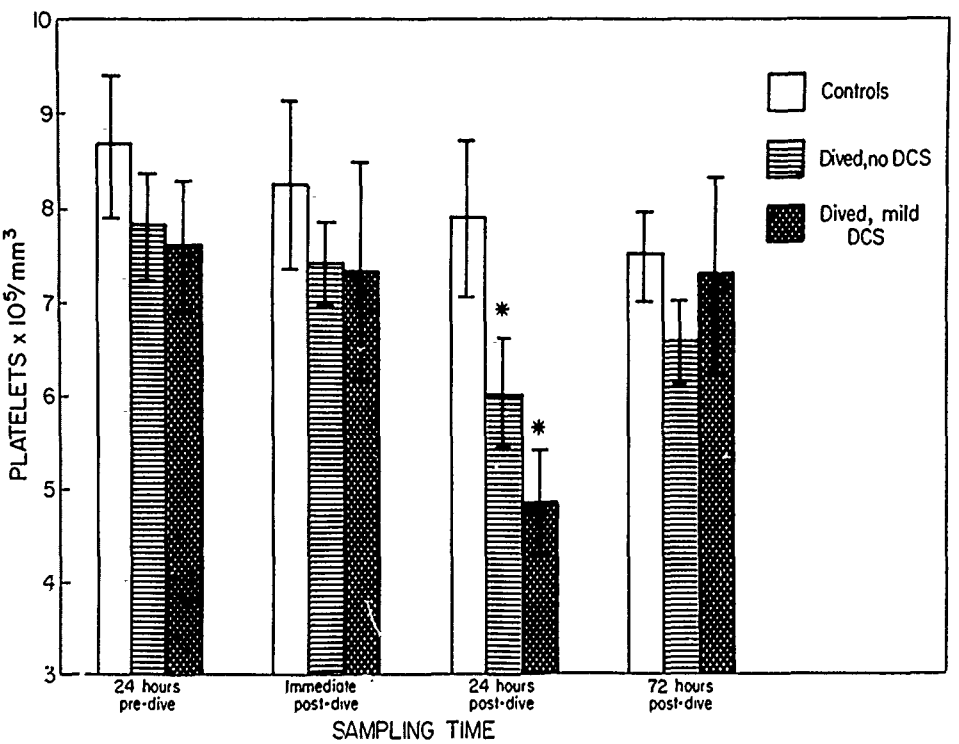

Fig. 1. Changes in peripheral platelet count with time in control and dived rats. The two instances where significant differences ($P < .05$) exist between control and dived animals are indicated by asterisks.

In the studies on drug prophylaxis for prevention or amelioration of DCS in the rat, neither PGE_1, Persantine, nor ASA was found to be effective under our experimental conditions. With PGE_1, dose levels as high as 5 mg/kg were administered subcutaneously in order to overcome the rapid rate with which the substance is cleared from the circulation by liver and kidney (Samuelsson 1964) and to maintain an inhibition of platelet aggregation several hours after injection. At this high dosage, however, a number of deleterious side effects produced by the drug rendered difficult a proper assessment of the material. As in other investigations using rats weighing in excess of 360 g (Clark, Philp, and Gowdey 1969; Inwood 1973), our results with animals of lighter weight support those conclusions regarding the inefficacy of ASA and Persantine as protective agents against DCS.

Although this communication does not provide direct confirmation of platelet-bubble interaction as the cause for the thrombocytopenic and altered platelet activity responses, it conforms well enough with observations made by other investigators to lend support to the contention that the bubble, be it silent or nonsilent, is the etiological factor responsible for the decompression effects (Philp 1974). It is apparent that the albino rat is a suitable laboratory model for investigating the effects of diving on blood components and should provide the means for pursuing future studies aimed at unraveling the multifaceted aspects of DCS.

We would like to thank Dr. John Pike of The Upjohn Company and Dr. M. Vinocur of Boehringer Ingelheim, Ltd., for generously supplying us with prostaglandin E_1 and Persantine, respectively; and Dr. Ray Fletcher for his helpful discussions on methodology during the course of the study.

This research was supported by the Bureau of Medicine and Surgery, Navy Department, Research Tasks MR011.0001|02.0031 and MPN 10.02.4080 BDK9. The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large. The animals used in this study were handled in accordance with the provisions of Public Law 89-544, as amended by Public Law 91-579, the "Animal Welfare Act of 1970" and the principles outlined in the *Guide for the Care and Use of Laboratory Animals,* U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

Received for publication November 1974.

Frattali, V., M. Quesada, and R. Robertson. 1975. Evidence d'une fonction plaquettaire altérée chez le rat après la plongée. Undersea Biomed. Res. 2(3):167-172.—Les modifications du fonctionnement plaquettaire et les variations de la population plaquettaire chez le rat de race Sprague-Dawley induites par un cycle de compression-décompression en N_2-O_2 capable de causer une maladie de décompression, ont été étudiées. Dans les cas ou des maladies de décompression modérées ont été produites, la réactivité plaquettaire à l'agrégation induite par ADP s'est manifestée immédiatement après la compression, et s'est trouvée accompagnée d'une inhibition accrue de l'agrégation par la prostaglandine E_1. Les deux phenomènes sont revenus aux valeurs témoins 24 heures après la plongée. Chez les animaux avec des maladies de décompression modérées, les numérations plaquettaires sont restées déprimées 24 heures après la plongée mais ont ressemblé aux valeurs temoins 72 heures après la plongée. Ces résultats tendent à renforcer les hypothèses actuelles sur la relation étiologique entre la coagulation intravasculaire disséminée et la maladie de décompression en fonction de nucleation de bulles.

syndrome de décompression-collapsus
plaquettes sanguines
numération plaquettaire
maladie de décompression
agrégation plaquettaire
rat

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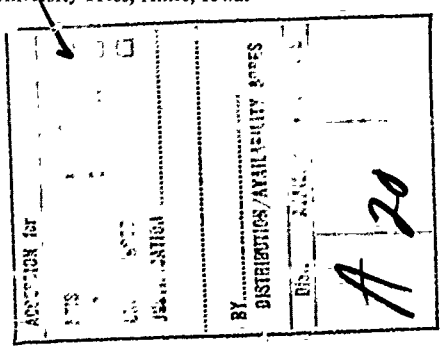

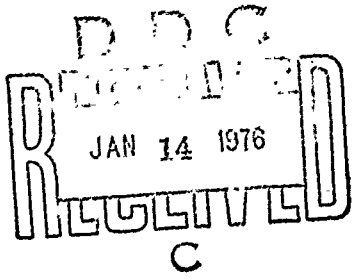

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
		(9)

4. TITLE (and Subtitle)

(6) SUPPORTIVE EVIDENCE FOR ALTERED PLATELET FUNCTION IN THE DIVED RAT.

5. TYPE OF REPORT & PERIOD COVERED

Medical Research Progress Report, ~~(publication)~~

~~6. PERFORMING ORG. REPORT NUMBER~~

7. AUTHOR(s)

(10) V. Frattali, M. Quesada, ~~[redacted]~~ R. Robertson

8. CONTRACT OR GRANT NUMBER(s)

(11) Nov-74

9. PERFORMING ORGANIZATION NAME AND ADDRESS

Naval Medical Research Institute
Bethesda, Maryland 20014

(12) 8p.

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

MPN10.02.4080
Report No. 6

11. CONTROLLING OFFICE NAME AND ADDRESS

Naval Medical Research & Development Command
Bethesda, Maryland 20014

12. REPORT DATE

September, 1975

13. NUMBER OF PAGES

6

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

Bureau of Medicine & Surgery
Department of the Navy
Washington, D. C. 20372

15. SECURITY CLASS. (of this report)

UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited

(16) MR011-00

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

(17) MR011-00-01

18. SUPPLEMENTARY NOTES

Undersea Biomedical Research, Vol. 2:167-172,
September 1975

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

decompression collapse syndrome	platelet aggregation
decompression sickness	platelet count
platelets	rat

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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S/N 0102-LF-014-6601

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